

A 3-bp Deletion in the Rhodopsin Gene in a Family with Autosomal Dominant Retinitis Pigmentosa

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Summary

Autosomal dominant retinitis pigmentosa (ADRP) has recently been linked to locus D3S47 (probe C17), with no recombination, in a single large Irish family. Other ADRP pedigrees have shown linkage at zero recombination, linkage with recombination, and no linkage, demonstrating genetic heterogeneity. The gene encoding rhodopsin, the rod photoreceptor pigment, is closely linked to locus D3S47 on chromosome 3q. A point mutation changing a conserved proline to histidine in the 23d codon of the gene has been demonstrated in affected members of one ADRP family and in 17 of 148 unrelated ADRP patients. We have sequenced the rhodopsin gene in a C17-linked ADRP family and have identified in the 4th exon an in-frame 3-bp deletion which deletes one of the two isoleucine monomers at codons 255 and 256. This mutation was not found in 30 other unrelated ADRP families. The deletion has arisen in the sequence TCATCAT-CAT, deleting one of a run of three \times 3-bp repeats. The mechanism by which this occurred may be similar to that which creates length variation in so-called mini- and microsatellites. Thus ADRP is an extremely heterogeneous disorder which can result from a range of defects in rhodopsin and which can have a locus or loci elsewhere in the genome.

Introduction

Retinitis pigmentosa (RP) is a term used to denote a collection of genetically determined disorders causing slow outer-retinal degeneration. Clinical characteristics include night blindness, constriction of visual fields, an abnormal fundus appearance, narrowed retinal vessels, and depression of the normal ocular electrophysiological responses (Merin and Auerbach 1976). The condition affects about 1/5,000 people in the United Kingdom, though estimates vary. RP can be further subdivided into autosomal dominant, autosomal recessive, and X-linked cases, which accounted for 22%, 10%, and 14% of patients, respectively in a recent survey (Bundey and Crews 1984). Of the remainder, 17% had autosomal recessive syndromes including RP, while 37%

were unclassified. Attempts have been made to subdivide ADRP further on the basis of age at onset, degree of gene penetrance, differential effects on rod and cone sensitivity, and distribution of pigment in the affected retina (Berson et al. 1969; Massof and Finkelstein 1979, 1981; Lyness et al. 1985). The now prevalent hypothesis subdivides ADRP into two categories, known as D type (diffuse) or type 1 ADRP, and R type (regional), or type II (Massof and Finkelstein 1981; Lyness et al. 1985). D-type ADRP leads to diffuse and severe loss of rod function early in life, but with relatively good preservation of cone function. Night blindness is consistently recognized before the age of 10 years, although pigmentary changes may not be evident until the second or third decade. The R-type disease shows patchy and equal loss of cone and rod function, with substantial variation in age at onset, both between and within affected families. Variants within the R-type category have been described with respect to variable expressivity, sectorial RP, and slow adaptation to darkness (Alexander and Fishman 1984; Fulton and Hansen 1988).

Linkage to locus D3S47 (probe C17) on chromosome 3q has recently been demonstrated in a single large Irish

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D-type ADRP family (TCDM1; McWilliam et al. 1989). The use of large families removes any problems associated with pooling lod scores in a heterogeneous disorder. The lod score of 14.7 with no recombination demonstrated very tight linkage. A further D-type family, ADRP3, has been shown to have linkage with no recombination (Lester et al. 1990), while two large R-type families have no linkage on chromosome 3q (Inglehearn et al. 1990; Lester et al. 1990). However, an R-type family has been shown to be linked at a recombination fraction of .08 (Olsson et al. 1990), raising the possibility of a second ADRP locus close by. The observed linkage led Dryja and co-workers to carry out genomic sequencing on the rhodopsin gene, also on 3q (Nathans et al. 1986), in ADRP patients. It was found that a CCC-to-CAC point mutation, changing a conserved proline to a histidine, was the likely cause of ADRP in about 12% of patients in a North American population (Dryja et al. 1990). We therefore analyzed the rhodopsin gene in two British families in which ADRP is known to be linked to C17. In one family (ADRP3, as described by Lester et al. 1990), we have found no mutations to date. In the other family (ADRP14), we identified a 3-bp deletion which is almost certainly the cause of ADRP in this pedigree.

Patients and Methods

Family ADRP14 shows linkage between the disorder and C17, with a lod score of 2.7 with no recombination. The disorder presents in early life, all affected members having had night blindness for as long as they could remember. Visual difficulties by day were not apparent until the third decade of life, and good visual acuity was retained until the seventh or eighth decade of life. Visual field restriction was apparent by the age of 20 years, but even in the seventh decade of life a scotopic field of 5°–10° was present. The ocular fundi showed irregularity of pigment in the retinal pigment epithelium, with areas of hypo- and hyperpigmentation. Pigment migration into the neuroretina was sparse even in late life. Patchy choroidal atrophy was evident by the age of 30 years and was widespread in the affected areas by the mid sixties. The macular appearance remained relatively normal into late life, with sharp demarcation between the affected and unaffected retina. These characteristics imply that the ADRP was class I or diffuse, although this has yet to be verified by psychophysical testing.

Genomic DNAs from members of affected pedigrees were prepared by a method based on that described by

Herman and Frischauf (1987). Amplification of rhodopsin exons by polymerase chain reaction (Saiki et al. 1988) was carried out using NBL *Taq* polymerase in the manufacturers buffer, with 30 cycles of 1 min at 91°C, 1 min at 55°C, and 2.5 min at 72°C. Oligomers used for amplification and sequencing of the exons were all 20-mers with 10 or more C/G residues, to give a T_d (dissociation temperature) equal to or greater than 60°C ($T_d = 4(G+C) + 2(A+T)$; Thein and Wallace 1986). Sequencing was carried out using combinations of oligomers such that the sequencing primer was different from those used to amplify and was therefore internal on the amplified molecule. Amplified sequences were treated with proteinase K, cleaned by extraction with phenol, phenol and chloroform, and chloroform, and then run through a Sepharose CL6B column (Pharmacia), according to a method described by Yandell and Dryja (1989). The primer was kinase end-labeled by the method of Chaconas and Van Der Sande (1980). Ten microliters of primer (2 pmol) and DNA (100–500 ng) were heated to 94°C for 3 min and then cooled on ice for 1 min. Sequencing was then carried out using T7 DNA polymerase (Sequenase) according to the manufacturer's (USB) instructions. Reactions were analyzed by separation on a 6% polyacrylamide wedge-sequencing gel. Dot blot analysis was carried out according to a method described elsewhere (Lester et al. 1990), with hybridization and washing at approximately 4°C below the melting temperature of the correctly aligned oligomer. The 100-bp PCR designed to visualize the deletion was performed as described above, but with 30 cycles of 30 s at 91°C, 30 s at 50°C, and 1 min at 72°C. Products of this PCR were visualized on a 10% polyacrylamide gel.

Results

Genomic sequencing of an affected member of family ADRP14 revealed a 3-bp deletion in the fourth exon of a patient from this family (fig. 1). This deletes one of two isoleucine monomers, at codons 255 and 256, from the sixth transmembrane hydrophobic segment of the rhodopsin molecule (fig. 2). We then synthesized 20-bp oligomers spanning codons 255 and 256, one oligomer with the normal and one with the mutant sequence. By hybridizing these oligomers separately to PCR-amplified fourth exon from all members of ADRP14, we demonstrated that the mutation is present in nine affected members and is absent in eight unaffected blood relatives (fig. 3). As further confirmation of the presence of a deletion in this family, oligos

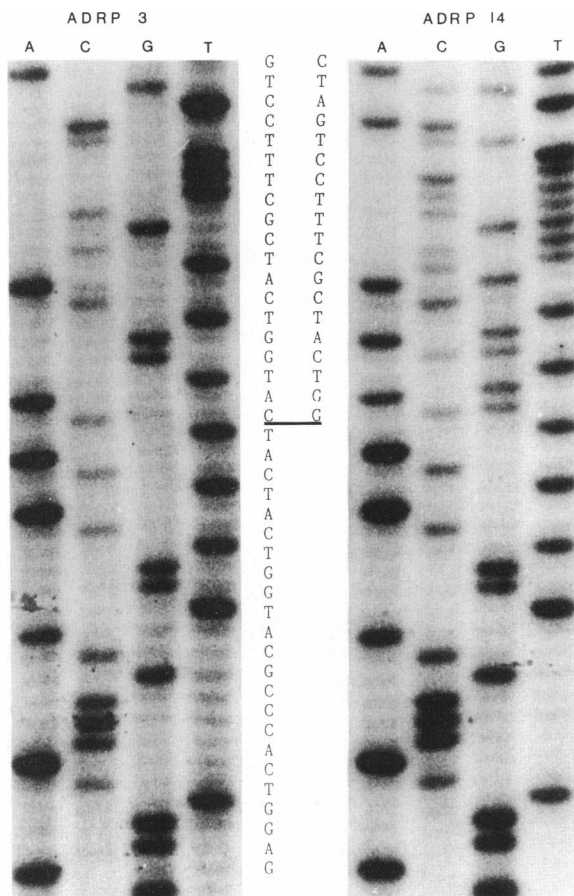


Figure 1 Sequencing gel showing sequence across point of deletion in family ADRP14, together with normal sequence, which is seen at this point in family ADRP3. The line in the written sequence at the center denotes the point from which the deletion is apparent. From that point upward, two sequences can be seen superimposed on one another, one being the correct one and the other being a 3-bp frameshifted one.

ACAGAAGGCAGAGAAGGAGG and GAAGATGTA-GAATGCCACGC, which flank the deletion, giving a normal PCR product of exactly 100 bp, were used to amplify DNA from normal and affected individuals (fig. 4). Normal individuals have only one band, while affected members can be seen to have a second band, 3-bp smaller than the normal sequence. In the same way we were able to exclude deletion or insertion in this part of the rhodopsin gene in a panel of 30 unrelated ADRP patients from affected pedigrees, four patients with other forms of inherited retinal degeneration, and 43 normal controls. Twenty-four of these families had previously been screened by oligo-hybridization for the presence of the codon 23 CCC→CAC

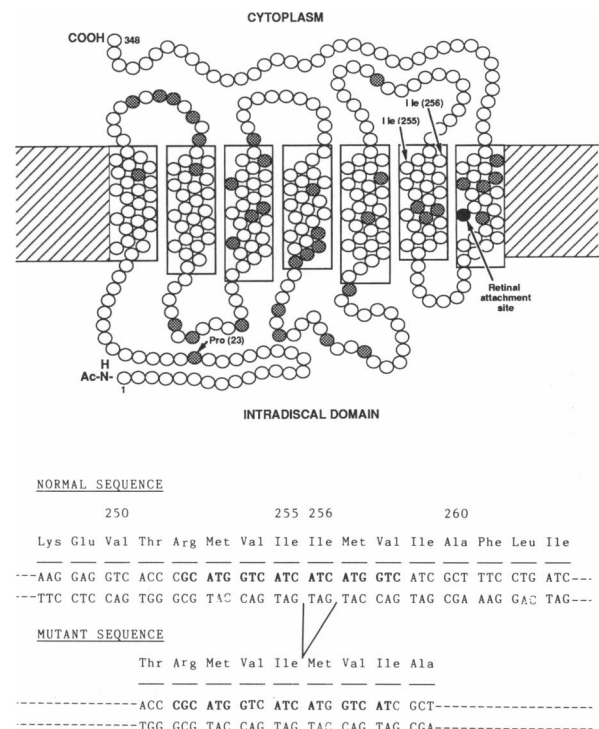


Figure 2 Top, Diagrammatic representation of rhodopsin molecule, showing location of codon 23 and codon 255 mutations. Shaded circles represent evolutionarily conserved amino acids (Applebury and Hargrave 1986). Bottom, Nucleotide and amino acid sequences of normal and mutant rhodopsin gene, at point of deletion. Oligomers used to detect the mutation are shown in boldface.

mutation, but none were found to have it (data not shown).

Discussion

Isoleucine residues at codons 255 and 256 are found in bovine and human rhodopsin but are not conserved among either the human color pigments or the visual pigments in other species (Applebury and Hargrave 1986). However, isoleucines 255 and 256 are common to several other receptor molecules which, like rhodopsin, are coupled to guanine nucleotide regulatory proteins (Kobilka et al. 1987). Since the mutation deletes an amino acid rather than substituting one, without changing the reading frame, it will have the effect of shortening the sixth transmembrane domain and may therefore affect both secondary structure and function of the gene product, beyond any significance which the deleted amino acid may have had. Clearly, then, this result, together with the previously reported codon 23

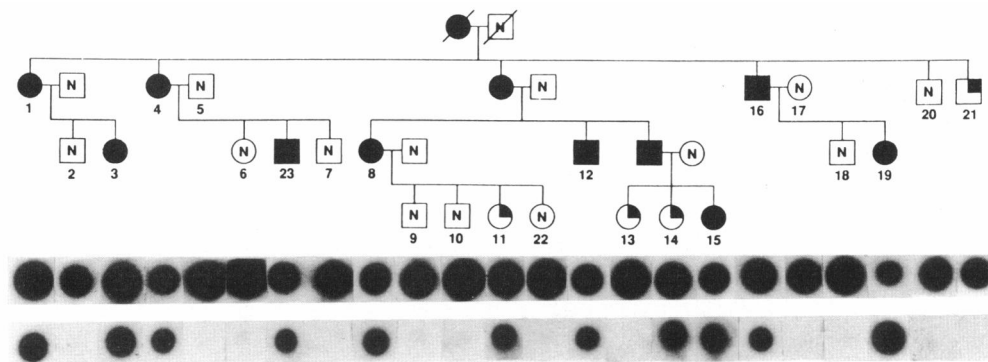


Figure 3 Dot blot hybridization of the normal (*upper row of gels*) and mutant (*lower row of gels*) oligomers (see fig. 2) to PCR-amplified fourth exon of rhodopsin from members of family ADRP14. The pedigree is shown above.

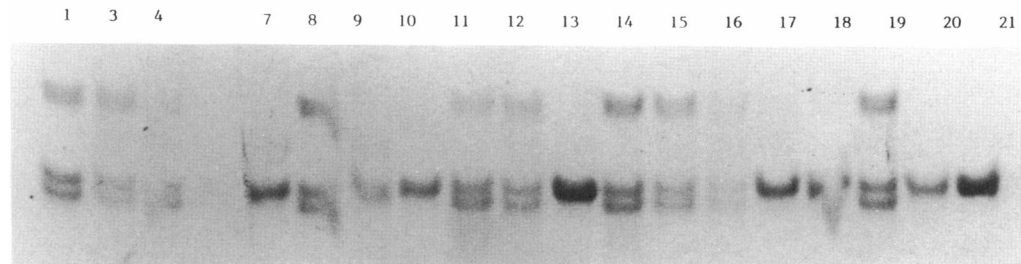


Figure 4 Polyacrylamide gel (10%) showing the products of a 100-bp PCR spanning the deletion in various members of family ADRP14. Numbers correspond to those on the pedigree shown in fig. 3. Normal individuals have only a slight band, while affected individuals have a doublet, as well as a third band above which is a heterodimer of the normal and mutant strands.

mutation, demonstrates that mutations in the rhodopsin gene can cause ADRP.

It is interesting to speculate on the mechanism by which this particular mutation arose. The deletion of 3 bp has occurred within the sequence 5'TCATCATCAT3'. Exactly which three nucleotides have been deleted is impossible to tell and would make no difference to the resultant mutation. It seems possible that the deletion occurred by a process similar to that which creates new length variants at microsatellite loci. Replication slippage at meiosis is a mechanism thought to be significant in tandem repeats of short (1–4-bp) motifs, though unequal crossover is also a possible cause (Jarman and Wells 1989). It would seem, therefore, that coding sequences are not exempt from the process by which such repeats arise.

In conclusion, consistent with observations on phenotype, ADRP is heterogeneous at every level, with (a) one or possibly two loci linked to C17 on chromosome 3q, (b) at least one other locus elsewhere on the autosomes, and (c) two or more different lesions within one

C17 linked gene, namely, rhodopsin. This is as might be expected for a dominant defect, since natural selection would prevent any one mutation from rising to a significant frequency in the population. That the codon 23 mutation does attain a significant frequency in a North American population could be due to repeated substitutions at a mutation hot spot. Alternatively, this mutation may have arisen only once but has remained in the gene pool for a significant time because the level of selection against it was not high—many patients retain adequate vision into later life.

Now that rhodopsin has been proved to be one location for genetic lesions causing RP, other molecules in the visual cascade are prime candidates in unlinked families, e.g., S-antigen on chromosome 2; transducin, encoded by genes on chromosomes 1 and 3; and retinal binding protein 2, also on chromosome 3 (Dancinger et al. 1989; Human Gene Mapping 10 1989). By identifying sites for ADRP mutations, it becomes possible to provide predictive testing with absolute certainty in some families. In addition, testing for specific defects

may allow counseling in isolated cases of RP, where linkage data are unavailable. Ultimately, knowledge of the underlying causes of RP which emerges from studies such as this, together with the steady increase in our understanding of the biochemical pathways of the healthy retina, may lead to therapies for this debilitating genetic disorder.

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